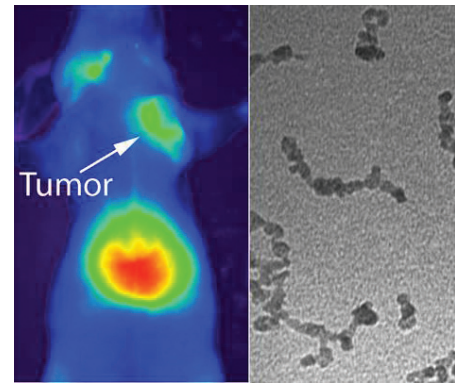
ANALYTICAL CURRENTS

## Improved superparamagnetic nanoparticles for targeting and imaging

Michael Sailor's group at the University of California San Diego (UCSD) and collaborators at the Massachusetts Institute of Technology and the University of California Santa Barbara have developed "nanoworms" (NWs) as a new tool for cancer bionanotechnology. NWs are elongated, dextran-coated nanostructures composed of iron oxide (IO) cores that are more effective in vitro and in vivo than traditional spherical IO nanoparticles.

Nanospheres (NSs) are 25–35 nm and made of one or two IO cores. NWs look like 50–80 nm polymer-coated strands of 5–10 pearls, in which each pearl is a 5 nm IO core. The researchers have not yet determined exactly how the NWs form or whether the IO cores touch each other, but they know that the elongated NW shape leads to longer $T_2$ magnetic relaxation times and allows for polyvalent binding to tumor cells in vitro.

To test the cellular uptake, both the NWs and NSs were derivatized with a peptide that targets tumor cells. When the two types of nanoparticles were incubated separately with human breast cancer cells in vitro, NWs showed greater internalization than NSs. In addition, unmodified NWs originating in the bloodstream and accumulating in the tumor stayed in the tumor tissue significantly longer than the NSs. Next, the researchers will apply the NW technology to develop in vivo nanoprobes for diagnosing and treating various human diseases. (*Adv. Mater.* **2008**, *20*, 1630–1635)




JI-HO PARK, UCSD

Left: Fluorescence image obtained 48 hours after injection of NWs into a mouse with a tumor. The NWs (bright green) can be seen in the tumor as well as the liver and the lymph nodes. Right: Transmission electron microscope image of the superparamagnetic IO NWs (~50 nm long).

## Spider on a chip

Here's a spider that won't frighten Miss Muffet away. Thomas Scheibel at the University of Bayreuth (Germany), Andreas Bausch at the Technical University of Munich, and colleagues created a microfluidic device that can assemble spider dragline silk proteins into fibers. The device permits the investigators to directly observe and identify the crucial factors in dragline silk assembly.

The development of an artificial method to produce spider silk strands—5× stronger than steel and therefore alluring as a material for a number of military and civilian applications—has been a frustratingly elusive goal. For instance, in 2002, the Canadian company Nexia demonstrated a way to cultivate silk proteins from transgenic goats but later abandoned the research. The in vivo process is so complicated that it has been hard to re-create the mechanism in vitro and better understand it.

Scheibel, Bausch, and colleagues fabricated a microfluidic device that mimicked the conditions in a spider's spinning duct. Two spider silk proteins were obtained from genetically engineered bacteria as the fiber precursors. The investigators introduced proteins and other requisite ionic and pH solutions into the device. Laminar flow and diffusion ensured that the correct ionic and pH conditions were present along the fiber assembly and elongation compartments for the two proteins to produce silk stands in aqueous conditions.

The investigators observed that colloidal aggregates of the proteins were a key step in fiber formation. The preaggregation produced micrometer-size particles, which were then needed for the hydrodynamic shear-induced fiber assembly. Once the particles come into contact, attractive interactions between them are critical for fiber formation. (*Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 6590–6595)

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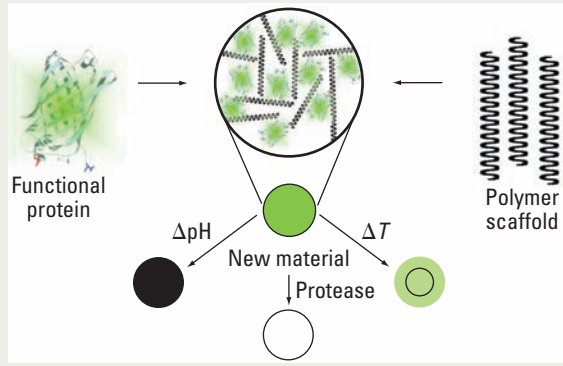
ANALYTICAL CURRENTS

## Green gels that swell

Because of their ability to change in response to properties such as temperature, pH, and ionic strength, protein–polymer hybrids could be used as sensors, drug-delivery systems, and nanomachine components. The hybrid syntheses proposed to date, however, have not been applicable to all proteins. To generalize protein–polymer hybrid production, Aaron Esser-Kahn and Matthew Francis at the University of California Berkeley recently presented a method for attaching polymers to both the C- and N-termini of any protein. They combined expressed protein ligation to modify the C-terminus and transamination of the N-terminus, yielding functional groups that would react with the methacrylamide polymer. The researchers demonstrated their new synthesis by using enhanced green fluorescent protein (EGFP) to produce a fluorescent hybrid hydrogel.




The unique properties of a material cross-linked by EGFP are illustrated: sensitivity to pH, heat, and enzyme degradation. (Adapted with permission. Copyright 2008 Wiley-VCH Verlag GmbH & Co. KGaA.)

Francis and Esser-Kahn hypothesized that the fluorescence and the size of the hydrogel should depend on the conformation of the protein. To test this theory, they first studied the swelling of the gel in buffer; it was stable for >24 hours (h). When they activated only one terminus of EGFP, the resulting hydrogel was not cross-linked. Once placed in the buffer, the resulting protein–polymer hybrid dissolved in 6 h. Because the cross-links in the material are formed only by the protein, the researchers expected the hydrogel to be rapidly biodegradable through enzyme degradation. When they added trypsin to swollen gels, the material disintegrated completely in <3 h.

Finally, the gel's sensitivity to pH and temperature was similar to that of free EGFP. Fluorescence decreased as the pH decreased from 6.5 to 5.5, and both fluorescence and volume decreased as temperatures rose above 60–65 °C. The authors note that the technique is somewhat labor-intensive but that its wide applicability outweighs this disadvantage. (*Angew. Chem., Int. Ed.* **2008**, *47*, 3751–3754)

## Lidar imaging of the Colosseum

The Flavian Amphitheater in Rome, more commonly known as the Colosseum, has endured almost two millennia of exposure to the elements and other harsh conditions, such as fire, and scavengers who take its materials for other construction projects. Dedicated in A.D. 80 and all but abandoned by the 5th century, the Colosseum received renewed interest in the 19th and 20th centuries. Numerous restoration projects—not all of them well-documented—were conducted during this period; it can be difficult to tell which parts of the monument have been restored and which are composed of the original building materials.

Now, Valentina Raimondi and colleagues at the Nello Carrara Institute for Applied Physics (Italy), Lund University (Sweden), and the Soprintendenza Archeologica di Roma have demonstrated that fluorescence lidar (equivalent to radar but with light waves) imaging can distinguish between original and restored areas of the Colosseum. The main advantages of lidar imaging, according to the researchers, are that it can be conducted from a distance and that it does not require the construction of scaffolding to analyze difficult-to-reach areas of a large structure like the Colosseum. It also avoids the need to collect samples from the structure for lab analysis.

The scientists looked at two different aspects of reconstruction: masonry repairs or replacements and strengthening elements, such as metal clamps, that were added to stabilize the structure over the years. After obtaining lidar spectra of the target areas, they used either principal component analysis or cluster analysis to show that they could distinguish between original and restored stone blocks and also pinpoint the location of metal clamps. The researchers note that their technique could easily be used for regular monitoring of any outdoor stone monument. (*Opt. Express* **2008**, *16*, 6794–6808)

A mobile lidar laboratory on-site at the Colosseum. (Adapted with permission. Copyright 2008 Optical Society of America.)

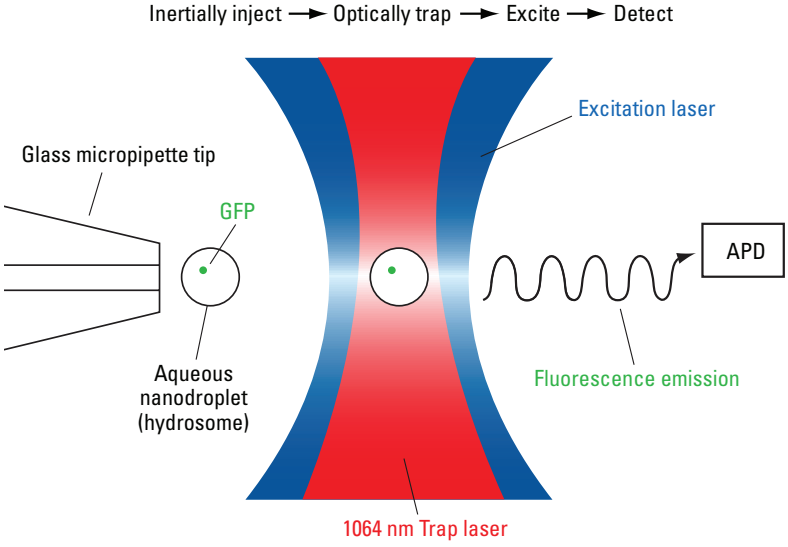
## Studying EGFP inside inertially injected droplets

Because droplet encapsulation might be a better model of biomolecular behavior in free solution and inside a crowded cell than surface immobilization, researchers have incorporated proteins and other molecules into aqueous droplets to study their dynamics. Now, by using an improved single-droplet injector, optical tweezers, and fluorescence measurements, Lori Goldner and her colleagues at the U.S. National Institute of Standards and Technology have studied the behavior of enhanced green fluorescent protein (EGFP) inside aqueous droplets, or hydrosomes.

Two methods of encapsulating molecules inside aqueous droplets involve ultrasonication, in which multiple droplets are created simultaneously, and microfluidics or some other means of creating droplets one at a time. On-demand single-droplet formation is a gentler technique, yields more reproducible droplet sizes, and consumes less sample.

In the new piezoelectric injector, a glass micropipette filled with aqueous solution quickly retracts, and the inertial force helps to create an aqueous droplet in the fluorocarbon continuous phase. After the hydrosome is created, optical tweezers trap it for fluorescence measurements. In one series of experiments, the group trapped hydrosomes containing single EGFP molecules and detected single-step photobleaching. Encapsulating the proteins in hydrosomes did not appear to damage the molecules or allow them to partition into the nonaqueous phase as long as surfactant was present.

The second set of experiments measured the rotational diffusion times by fluorescence anisotropy and concluded that, in the presence of surfactant, EGFP did not accumulate at the interface between the two phases and that the rotation of EGFP inside a hydrosome was similar to its rotation in free solution. The team suggests that its method could also be used to study complex formation. (*Langmuir* **2008**, *24*, 4975–4978)




Schematic of hydrosomes injected inertially into a fluorocarbon matrix by a piezoelectric injector. After excitation of the EGFP molecules in the hydrosomes, fluorescence is detected with an avalanche photodiode (APD).

## A monomeric alternative to α-hemolysin

Historically, the biological nanopore field has relied on α-hemolysin (α-HL), a multisubunit β-barrel pore, for sensing. OmpG is an alternative monomeric pore that would be simpler and easier to modify than α-HL. It has been pursued as an alternative for sensing, but at neutral pH, it exhibits spontaneous gating that makes application as a biosensor nearly impossible. Now, Hagan Bayley and colleagues at the University of Oxford (U.K.) have engineered OmpG to quiet its "chatter".

The researchers started by examining the crystal structure of OmpG and performing molecular dynamics simulations to identify which regions of the protein were involved in its gating behavior. They found that loop 6 (L6) of strand β12 was partially unfolded in the closed state, blocking the pore opening. They hypothesized that if they could stabilize L6, the pore would remain open for longer periods of time.

The group took a 2-fold approach: first, they created a disulfide bond between β12 and the adjacent β13 strand and then they optimized hydrogen bonding between strands β11 and β12 by deleting an aspartate residue. By combining these mutations into one protein, the researchers could reduce the gating frequency of the mutant (qOmpG) to <4% of that of the wild-type protein.

Finally, as a proof of principle for sensing, qOmpG was combined with a cyclodextrin molecular adapter and used to detect adenosine diphosphate (ADP). The scientists found that the residence time of ADP in the modified pore was 0.42 ms—long enough to identify and quantify ADP. (*Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 6272–6277)

ANALYTICAL CURRENTS

## A fast, label-free nanofluidic DNA detector

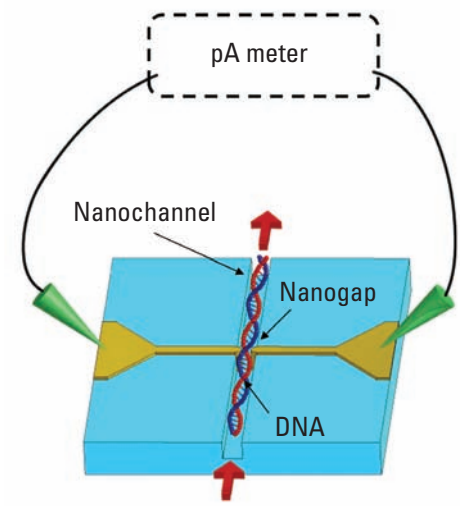
Sequencing individual DNA molecules is one of the lofty goals of modern biology. Stephen Chou and Xiaogan Liang at Princeton University have fabricated a nanofluidic DNA detector that measures the electrical conduction of single DNA molecules as they pass through a nanometer-scale gap. Theoretical studies suggest that a tunneling current perpendicular to a DNA backbone is sensitive enough to distinguish single base types for sequencing.

Nanopore detectors are an alternative approach to single-molecule sequencing but are hampered by two obstacles. The first is the poor confinement of a DNA strand in a nanopore; the poor confinement results in random motion of DNA in solution that creates a lot of noise and overwhelms a single base's signal. The second obstacle is the signal sensitivity—it is uncertain whether a nanopore detector is sensitive enough to recognize two adjacent bases on a DNA molecule.

To bypass these challenges, Chou and Liang fabricated a long nanofluidic channel that stretched a DNA molecule into a straight chain. A pair of metal nanowires with a gap between them measured the electrical conduction as the DNA passed through the gap.

The investigators tested different dimensions with the nanowires and the channel. For a detector with a gap of 18 nm between the nanowires and a channel 35 nm high, no noticeable difference was measured in electrical current signals between the pure buffer solution and the DNA solution. But as the dimensions were reduced to a 13 nm gap and a 26 nm height, clear negative pulses were observed only with the DNA solution. When dimensions were reduced to a 9 nm gap and a 16 nm height, the average magnitude of negative pulses increased by ~200%.

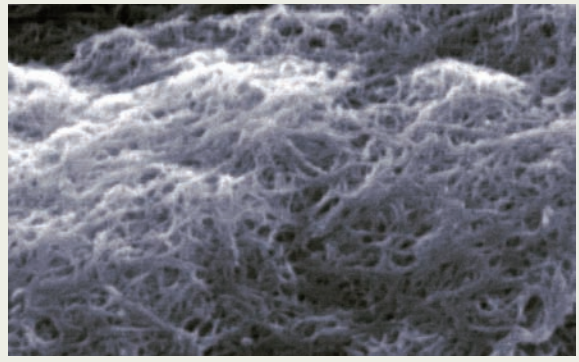
Chou and Liang note they have not yet resolved single DNA bases. They aim to do so by further reducing the dimensions of the detector, slowing down the DNA's flow, and cutting down on fluctuations in the DNA's translocation time. (*Nano Lett.* **2008**, *8*, 1472–1476)




The detector consists of a nanofluidic channel in which a DNA molecule is stretched into a linear chain. A pair of metal nanowires with a gap as small as 9 nm measures electrical conduction perpendicular to the DNA backbone as the DNA moves through the gap.

## New guidelines for SWNT measurements

The U.S. National Institute of Standards and Technology (NIST), in collaboration with NASA, recently issued detailed guidelines for making measurements on single-walled carbon nanotubes (SWNTs). Because of their unique electronic, thermal, optical, and mechanical properties, SWNTs are being studied for a wide range of applications, such as circuit elements in molecular electronics. However, making pure, high-quality SWNTs is difficult because current synthesis methods produce a byproduct of small graphite and metal particles. Therefore, accurate, reliable, and rapid measurement techniques are needed to optimize the production of SWNTs and to give a greater yield with a higher purity. The "Recommended Practice Guide" is a product of the NIST/NASA series of workshops on nanotube measurements, which started in 2003. This NIST special publication is part of the "How to Measure" book series (www.nist.gov/public_affairs/practiceguides/practiceguides.htm), which was created to teach people how to measure properly, what to measure, which techniques to use, and how to interpret results. Other topics in the book series include surface engineering, X-ray topography, and particle size characterization.


NIST

SEM image of "cleaned" SWNTs (color added for clarity).